

AMENDMENTS TO THE CLAIMS

1. (currently amended): A process for preparing a vascular endothelial growth factor (VEGF) dimer comprising:

providing transformed host bacterial cells, wherein the transformed host bacterial cells comprise an exogenous nucleic acid encoding an amino acid sequence of a VEGF monomer operably linked to a promoter, wherein the amino acid sequence has at least about 90% sequence identity with amino acids 11 to 116 of ~~SEQ ID NO: 1~~ SEQ ID NO: 2 and wherein the amino acid sequence is extended by a Met-(AA)_n- sequence at the amino terminus (N-terminus), wherein Met stands for methionine, n is 1-7, and AA represents identical or different amino acids, where at least one of the AA amino acids, or a combination of two or more of the AA amino acids, is capable of retarding proteolytic degradation of the mature N-terminus of the VEGF dimer by the bacterial host cell, and the amino acid sequence retains a cysteine (Cys) at ~~or corresponding to~~ position 116 of ~~SEQ ID NO: 1~~ SEQ ID NO: 2 (Cys-116);

culturing said host cells under conditions suitable for expression of said VEGF monomer, whereby a first VEGF monomer and a second VEGF monomer are produced;
forming the VEGF dimer from the first and second VEGF monomers; and
recovering said VEGF dimer.

2. (original): The process of claim 1, wherein n is 1.

3. (original): The process of claim 2, wherein AA represents an amino acid selected from the group consisting of lysine (Lys) and arginine (Arg) residues.

4. (original): The process of claim 3, wherein AA represents a lysine (Lys) residue.

5. (original): The process of claim 1, further comprising the step of purifying said VEGF dimers.

6. (original): The process of claim 5, further comprising the removal of the N-terminal Met(AA)_n- sequence following at least partial purification.

7. (original): The process of claim 6, wherein removal is performed by enzymatic digestion.

8. (original): The process of claim 7, wherein diaminopeptidase is used to perform the enzymatic digestion.

9. (canceled)

10. (original): The process of claim 1, further comprising the step of refolding said VEGF dimers.

11. (currently amended): The process of claim 10, wherein refolding is performed in a refolding buffer comprising cysteine and cystine in amounts and in a ratio to each other sufficient to produce ~~the desired~~ a mixture of VEGF dimers.

12. (currently amended): A process for producing a vascular endothelial growth factor (VEGF) dimer composed of two VEGF monomers, in which each monomer comprises amino acids 11 to 116 of ~~SEQ ID NO: 1~~ SEQ ID NO: 2, or comprises an amino acid sequence having at least about 90% sequence identity with amino acids 11 to 116 of ~~SEQ ID NO: 1~~ SEQ ID NO: 2, and retaining a cysteine (Cys) at ~~[[a]]~~ position ~~corresponding to position~~ 116 of ~~SEQ ID NO: 1~~ SEQ ID NO: 2 (Cys-116), where Cys-116 of each monomer is disulfide bonded to an additional extraneous Cys, comprising the steps of:

providing transformed bacterial host cells comprising a species of exogenous nucleic acid encoding a promoter operably linked to a polypeptide of ~~SEQ ID NO: 1~~ SEQ ID NO: 2 extended by a Met-(AA)_n- sequence at the amino terminus (N-terminus), wherein Met stands for methionine, n is 1-7, and AA represents identical or different amino acids, wherein at least one of the AA amino

acids, or a combination of two or more of the AA amino acids, is capable of blocking the proteolytic degradation of the mature N-terminus of the VEGF polypeptides by the bacterial host cell;

culturing said bacterial host cells under conditions suitable for expression of said exogenous nucleic acid and the synthesis of said N-terminally-extended VEGF monomers[.,.];

disulfide bonding the Cys-116 of each monomer to the extraneous Cys;

forming the dimer; and

recovering said VEGF dimer.

13. (original): The process of claim 12, wherein n is 1.

14. (original): The process of claim 13, wherein AA represents an amino acid selected from the group consisting of lysine (Lys) and arginine (Arg) residues.

15. (original): The process of claim 14, wherein AA represents a lysine (Lys) residue.

16. (original): The process of claim 12, further comprising the step of purifying said VEGF dimer.

17. (original): The process of claim 16, further comprising the removal of the N-terminal Met(AA)_n- sequence following at least partial purification.

18. (original): The process of claim 17, wherein removal is performed by enzymatic digestion.

19. (original): The process of claim 18, wherein at least about 95% of said VEGF dimers are devoid of an N-terminal methionine residue.

20. (original): The process of claim 12, additionally comprising the step of refolding said VEGF dimer.

21. (original): The process of claim 14, additionally comprising the step of refolding said VEGF dimer.

22. (original): The process of claim 17, additionally comprising the step of refolding said VEGF dimer.

23. (original): The process of claim 22, wherein refolding is performed in a refolding buffer comprising cysteine and cystine.

24. (currently amended): A process for preparing a vascular endothelial growth factor (VEGF) dimer comprising:

providing host cells, wherein the host cells comprise an exogenous nucleic acid encoding an amino acid sequence of a VEGF monomer operably linked to a promoter, wherein the amino acid sequence has at least about 90% sequence identity with amino acids 11 to 116 of ~~SEQ ID NO: 1~~ SEQ ID NO: 2, retains a cysteine (Cys) at ~~or corresponding to~~ position 116 of ~~SEQ ID NO: 1~~ SEQ ID NO: 2 (Cys-116), and wherein at least one monomer has an Asn-to-Glu amino acid substitution at ~~or corresponding to~~ position 75 of ~~SEQ ID NO: 1~~ SEQ ID NO: 2;

culturing said host cells under conditions suitable for expression of said VEGF monomer, whereby a first VEGF monomer and a second VEGF monomer are produced;
forming the VEGF dimer from the first and second VEGF monomers; and
recovering said VEGF dimer.

25. (currently amended): The process of claim 24, wherein each monomer comprises amino acids 1 to 120 of ~~SEQ ID NO: 1~~ SEQ ID NO: 2.

26. (currently amended): The process of claim 24, wherein monomer comprises amino acids 1 to 121 of ~~SEQ ID NO: 1~~ SEQ ID NO: 2.

27. (canceled)

28. (currently amended): The process of claim 24, further comprising a bonding step, wherein the ~~Cys residue corresponding to~~ Cys-116 of ~~SEQ ID NO:1~~ SEQ ID NO:2 of each monomer is disulfide bonded to an extraneous Cys.

29. (currently amended): The process of claim 24, wherein the ~~Cys residue corresponding to~~ Cys-116 of ~~SEQ ID NO:1~~ SEQ ID NO:2 of the two monomers are interconnected with an interchain disulfide bond.

30. (currently amended): The process of claim 24, wherein the ~~Cys residue corresponding to~~ Cys-116 of ~~SEQ ID NO:1~~ SEQ ID NO:2 of one or both monomers is not reduced.

31. (original): The process of claim 24, additionally comprising the step of purifying said dimers.

32. (original): The process of claim 24, wherein said transformed host cells are bacterial cells.

33. (original): The process of claim 32, wherein said bacterial cells are *E. coli* cells.

34. (currently amended): The process of claim 32, wherein the exogenous nucleic acid encodes a polypeptide of ~~SEQ ID NO:1~~ SEQ ID NO: 2 extended by a Met-(AA)_n- sequence at the amino terminus (N-terminus), wherein Met stands for methionine, n is 1-7, and AA represents identical or different amino acids, where at least one of the AA amino acids, or a combination of two or more of the AA amino acids, is capable of retarding proteolytic degradation of the mature N-terminus of the VEGF dimer by the bacterial host cell.

35. (original): The process of claim 34, wherein n is 1.

36. (original): The process of claim 35, wherein AA represents an amino acid selected from the group consisting of lysine (Lys) and arginine (Arg) residues.

37. (original): The process of claim 36, wherein AA represents a lysine (Lys) residue.
38. (original): The process of claim 34, further comprising the step of purifying said VEGF dimers.
39. (original): The process of claim 38, further comprising the removal of the N-terminal Met(AA)_n- sequence following at least partial purification.
40. (original): The process of claim 39, wherein removal is performed by enzymatic digestion.
41. (original): The process of claim 32, further comprising the step of refolding said VEGF dimers.
42. (original): The process of claim 41, wherein refolding is performed in a refolding buffer comprising cysteine and cystine in amounts and in a ratio to each other sufficient to produce the desired mixture of VEGF dimers.